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Heamato-immunological and physiological responses of *Labeo rohita* fingerlings to dietary fermented *Jatropha curcas* protein concentrate

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ABSTRACT

Jatropha protein concentrate (JPC) prepared from jatropha seed cake is toxic due to the presence of phorbol ester and antinutritional factors like tannin, trypsin inhibitor, phytate and non-starch polysaccharides. JPC was detoxified by solid state fermentation (SSF) and a feeding trial of 45 days was conducted to study the response of feeding fermented JPC (FJPC) on growth, haemato-immunological and physiological responses in rohu fingerlings. Seven iso-nitrogenous diets such as control (without JPC or FJPC), J5 (5% JPC), J10 (10% JPC), J20 (20% JPC), FJ5 (5% FJPC), FJ10 (10% FJPC) and FJ20 (20% FJPC) were prepared and fed twice daily. The weight gain and specific growth rate values showed an overall, linear and quadratic trend with similar value recorded in the control and FJ fed groups. Feed efficiency also showed an overall significant effect with a higher feed efficiency value recorded in the control (60.50) group which was similar to FJ (54.21–58.37) fed groups, while JPC fed groups registered the lowest value. The haematological studies showed a significantly ($p < 0.05$) lower red blood cells (RBC) and heamoglobin in all JPC fed groups and 5% FJPC group compared to control and other FJPC groups. The 10% and 20% JPC fed groups showed the highest blood glucose level than any other groups. Serum total protein and albumin followed similar trend as that of RBC and haemoglobin. The highest globulin value were observed in FJ10 group which was significantly different ($P < 0.05$) to other groups and lowest value was recorded in J20 group. The superoxide dismutase (SOD) activity in liver was higher in J20, while in muscle, J10 and J20 registered the highest value compared to other groups ($p < 0.05$). Our study revealed that protein concentrate prepared from Jatropha cake cannot be fed directly to rohu without detoxification and solid state fermentation appears to be an ideal approach. Overall, FJPC can be utilized in the diet of rohu at 20% without any detrimental effect on heamato-immunological and physiological response.

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1. Introduction

Several alternative plant protein source ingredients have been studied over the last few decades due to the reduction in fish meal production and increasing cost of fish meal in the industry (Jackson, 2006; Hardy, 2010). Soybean meal (SBM) is one of the most studied plant protein source and are considered as a good ingredient for aquafeed production (Kaushik et al., 1995). However, competition with human food and high demand in other feed industry has led to a hike in the price of soybean meal (FAO, 2006). Also, Ray et al. (2013) reported that the yield trends of most of the crops like rice, soybean, groundnut etc., are insufficient to meet the human requirement in the coming years. Hence, it is imperative to explore an alternative, less expensive, non-edible ingredient to satisfy the aquaculture requirement. One of the best alternatives in this regard is to utilize the industrial wastes and by-products which are cheap, surplus and eco-friendly. Global oil industry is now attempting to utilize non-edible seeds like rubber seeds, neem seeds, pongamia, karanja, mahua, Jatropha etc., for biodiesel production as there is debate in utilizing edible seeds for the same (Butler, 2006; Meher et al., 2006). A promising candidate of this industry is *Jatropha curcas* (family Euphorbaceae), a drought resistant tropical shrub. Large-scale Jatropha cultivation projects of about 12.8million hectares yielding 2 t/ha of oil by 2015 has been initiated worldwide (Global Exchange for Social Investment market study GEXSI, 2008). The main by-product of bio-diesel industry utilizing jatropha is Jatropha Seed Cake (JSC) and its protein content ranges from 27 to 33% (Makkar and Becker, 2008). As JSC contains less protein, the better way to enhance its utilization is to convert them into a protein concentrate in line with soybean protein concentrate. Protein concentrate contain high protein and low fibre content. There are several method for the preparation of protein concentrate like iso-electric precipitation (Devappa and Swamylingappa, 2008), alkaline extraction (Tobin and Carpenter, 1978), sedimentation-flotation (Parrado et al., 1991), and ultra-filtration method (McClements, 2013). Different research works were conducted recently to study the inclusion level of various protein concentrate in fish like canola protein concentrate in atlantic salmon and rainbow trout (Burr et al., 2013; Slawski et al., 2013), Pea protein concentrate in juvenile crayfish (Fuertes et al., 2014), and soybean protein concentrate in African catfish and *Tinca tinca* (Rodríguez et al., 2014). With this backdrop, we prepared JPC using iso-electric precipitation method. Even though the extraction itself reduced the ANFs, the presences of phorbol ester still remain high above the tolerance level (Shamna et al., 2015). In the present study we detoxified JPC using solid state fermentation (SSF) and studied the effect of JPC and FJPC on the growth indices, hemato-immunological parameters and stress enzyme activities in *Labeo rohita* fingerlings.

2. Materials and methods

2.1. Preparation and detoxification of jatropha protein concentrate

Jatropha protein concentrate (JPC) was prepared as described in the complementary work by Shamna et al. (2015). JPC was detoxified by solid state fermentation, in summary, *Aspergillus niger*(MTCC 281) obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India, was subculture in Czapek Yeast Extract Agar (CYA) and the fungal spores were collected in Tween-80 solution and diluted to get 10^7–10^8 spores mL^{-1} by serial dilution. Fermentation was conducted for a period of seven days on dried JPC at 30° C aerobically.

2.2. Diet formulation

Seven iso-nitrogenous (about 340 g/ kg crude protein) experimental diets were formulated (Table 1). Control diet contained soy protein concentrate (SPC) as major protein source and devoid of JPC and FJPC, whereas the treatment groups contained 50, 100 and 200 g/ kg JPC or FJPC, replacing equal amount of SPC in the diet. Other ingredients like fishmeal, groundnut oil cake, rice flour, wheat flour, corn flour were identical for all the diets. Oil and vitamin-mineral mixture were included in all the diets at equal level. Carboxymethyl cellulose (CMC) was used as binder and butylated hydroxyl toluene (BHT) was used as antioxidants in the diets.

2.3. Experimental set-up and sampling

The experiment was conducted in 21 plastic rectangular tubs (80 × 57 × 42 cm, 150 L capacity) covered with perforated lids previously treated and cleaned with potassium permanganate solution (5 ppm). Two hundred and ten fingerlings (initial weight 6.35 ± 0.02 g) were randomly distributed in seven distinct experimental groups like Control; J5 (50 g/ kg JPC); J10 (100 g/ kg JPC); J20 (200 g/ kg JPC); FJ5 (50 g/ kg FJPC); FJ10 (100 g/ kg FJPC) and FJ20 (200 g/ kg FJPC) in triplicates. The experiment was conducted for a period of 45 days and feeding was done to satiation level. Fishes were starved overnight before taking the body weight at every 15 days. Mortality was recorded throughout the experiment. After 45 days of feeding, six fishes were sampled from each tank with a total of 18 from each treatment and anesthetized using CIFECALM (CIFE, Mumbai, India) at 200 μl/ L. Tissues of different organs (liver and muscle) were dissected out and homogenized with 0.25 M chilled sucrose solution in a glass tube using teflon coated mechanical tissue homogenizer. The tube was continuously kept in ice to avoid heating. The homogenate was centrifuged at 5000 rpm for 10 min in a cooling centrifuge (REMICPR-24, India). The

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